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REVIEW

The Potential of Exosomes for Osteoporosis Treatment: A Review

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Abstract: As a continuous process comprising bone resorption and formation, bone remodeling, plays an essential role in maintaining the balance of bone metabolism. One type of metabolic osteopathy is osteoporosis, which is defined by low bone mass and deteriorating bone microstructure. Osteoporosis patients are more likely to experience frequent osteoporotic fractures, which makes osteoporosis prevention and treatment crucial. A growing body of research has revealed that exosomes, which are homogenous vesicles released by most cell types, play a major role in mediating a number of pathophysiological processes, including osteoporosis. Exosomes may act as a mediator in cell-to-cell communication and offer a fresh perspective on information sharing. This review discusses the characteristics of exosomes and outlines the exosomes' underlying mechanism that contributes to the onset of osteoporosis. Recent years have seen a rise in interest in the role of exosomes in osteoporosis, which has given rise to innovative therapeutic approaches for the disease prevention and management.

Keywords: exosomes, extracellular vesicles, osteoporosis, osteoblast function, osteoclast function

Introduction

Extracellular vesicles (EVs) have been examined extensively, and exosomes are one such kind. Almost all types of cells release EVs, precisely, tiny, submicron-sized lipid-bilayer-enclosed vesicles, into the extracellular environment.[1] EVs can be categorized into three primary groups based on their size, structure, and biogenesis: exosomes (30–200 nm in diameter), microvesicles (100–1000 nm in diameter), and apoptotic bodies (1–5 μm in diameter).[2] Every type of EV has a unique process of formation. When multivesicular bodies (MVBs) fuse with the plasma membrane, the inward budding of the MVBs' membrane generates homogenous vesicles called exosomes, which are subsequently released into the extracellular environment.[3] Microvesicles (MVs), which originate from direct protrusions of the cell membrane that detach from the surface, are directly released into the extracellular environment.[4] Apoptotic bodies are generated by shedding cells when the cells undergo apoptosis[5] (Figure 1).

Exosomes were first discovered in the in vitro culture of sheep reticulocytes.[6,7] Exosomes can be secreted by nearly all mammalian cell types (such as endothelial cells, neuronal cells, muscle cells, hematopoietic cells, and various tumor cells) in physiological and pathological conditions.[8] Various biological fluids, for instance, plasma, serum, urine, cerebral spinal fluid, amniotic fluid, saliva, semen, and breast milk naturally contain exosomes.[9] The exosomes display pronounced molecular heterogeneity, mainly including their size, content, cellular origin, and functional impact on recipient cells.[10] The heterogeneity of exosomes might be an outcome of cellular origin, uneven invagination of MVBs, metabolic status, and the microenvironment of cells.[11] Initially underestimated as molecular garbage bins, exosomes have gained considerable attention as a novel and potent mechanism of intercellular communication.[12] Exosomes can transfer biological signals to modulate target cell biology and function in physiological and pathological processes.[13] Exosomes can carry diverse constituents, including nucleic acids (DNA, mRNA, and noncoding RNA), proteins, lipids, amino acids, and metabolites to promote intercellular communication.[10] Exosomes have diverse effects on basic

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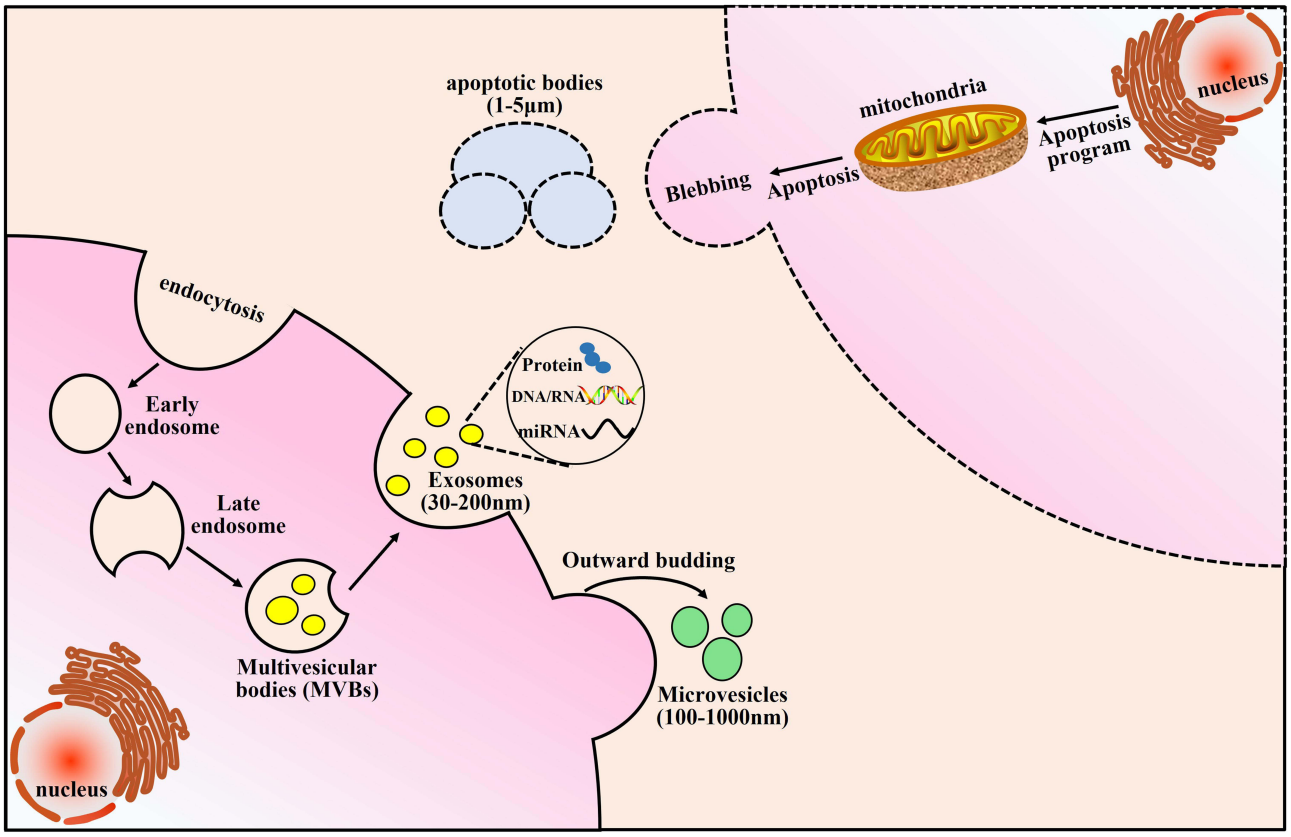

Figure 1 Schematic representation of the biogenesis of extracellular vesicles (EVs). EVs are formed according to mechanisms specific to the type of EVs. Exosomes (30–200nm) are generated by intraluminal buds fusing with the cell membranes. Initially, the cell membrane invaginates or endocytose to form the early endosome. Early endosomes invaginates to form late endosomes which incorporates endocytic vesicles. Then, the late endosomal membrane invaginates to generate intraluminal vesicles in the lumen of the multivesicular bodies (MVBs). The exosomes are released into extracellular space through exocytosis when the MVBs fuse with the plasma membrane. Microvesicles (100–1000nm) are generated directly from outward budding of the cell membrane. Apoptotic bodies (1–5μm) are directly generated by outward blebbing from apoptotic cells.

biological processes that are mediated in pleiotropic ways. For example, they can fuse their membrane contents into the plasma membrane of the recipient cell, cause direct activation of cell surface receptors through bioactive lipid and protein ligands, and deliver effectors such as oncogenes, transcription factors, and short and long non-coding regulatory RNAs into the recipient cells.[14]

Bone tissue, containing collagen and calcium phosphate is a kind of mineralized connective tissue, that is crucial to the musculoskeletal system and gives tendons and ligaments structural support. The balance of bone metabolism is dependent upon bone remodeling, which is a constant process of new bone synthesis by osteoblasts and old bone resorption by osteoclasts.[15] A systemic skeletal illness called osteoporosis (OP) is characterized by the weakening of the bones, which increases the risk of fractures.[16] Besides their role in a variety of biological processes and illnesses, exosomes have also been shown to be significant in osteoporosis.[17,18]

This review primarily focuses on the relationship between the exosomes and the pathogenesis of osteoporosis. Moreover, the applications of exosomes in preventing and treating osteoporosis have been summarized, which may provide a new treatment paradigm for osteoporosis.

Basic Biology of Bone Remodeling

The appropriate combination of bone production and bone resorption results in normal physiological bone remodeling. The osteoblast lineage, which includes osteoblasts, osteocytes, and bone lining cells, as well as the bone-resorbing cells (osteoclasts), are the primary cells engaged in bone remodeling. Bone multicellular units (BMUs) are specialized units made up of these cells and their precursor cells.[19] Bone lining cells are a thin layer of post-mitotic flat osteoblast lineage

cells that cover the surface of the bone, while they are quiescent. It is possible to stimulate the growth and differentiation of bone lining cells into osteogenic cells, which could serve as a source of "determined" osteogenic precursors.[20] Mesenchymal stem cells (MSCs) within the bone marrow stroma are the source of pluripotent osteoblasts, which are specialized bone-forming cells. Afterwards, osteoprogenitors are produced by MSCs. The osteoprogenitors divide and multiply into preosteoblasts that proliferate. Preosteoblast eventually develops into osteoblast. As the committed osteoblast becomes less active, the neighboring osteoblasts bury it further into the bone matrix. Certain osteoblasts are capable of differentiating into osteocytes. The last stage of development in the osteoblast lineage of mesenchymal stem cells is the mature osteocytes or stellate cells. The precise location of the osteocytes is inside the hard, mineralized matrix, within the lacuna. The remaining osteoblasts undergo apoptosis after completing the mineralization process.[21]

The multinucleated cells known as osteoclasts, which degrade bone, are produced from hematopoietic stem cells (HSCs) found within the bone marrow. The stromal cells generate M-CSF (macrophage colony-stimulating factor) and RAKL (receptor activator of the NF-κB ligand), which are known to stimulate osteoclastogenesis. HSCs, in the presence of M-CSF, are steered toward macrophage colony-forming units (CFU-M), which are precursors of osteoclast.[22] The receptor RANK, which is mostly expressed in immune cells, mature osteoclasts, and osteoclast precursors, is bound by RANKL.[23] This results in the differentiation of osteoclast precursors into mononucleated osteoclasts, which then fuse to become multinucleated osteoclasts, and trigger the activation of osteoclasts.[24]

The Potential Therapeutic Effects of EVs in Osteogenic Differentiation

EVs have the potential to treat osteoporosis because they can contain proteins, long non-coding RNA (lncRNA), microRNAs (miRNAs), and mRNAs. EVs are able to deliver this payload to nearby or far-off cells and cause biological reactions that correspond to the contents of the cargo.

Bone Marrow Mesenchymal Stem Cells (BMSCs)-Derived Exosomes

The major progenitors of bone marrow adipocytes and osteoblastic-lineage cells are bone marrow mesenchymal stem cells (BMSCs). Strict spatiotemporal constraints govern the reciprocal balance between BMSCs' osteogenic and adipogenic development to preserve skeletal health. The equilibrium between osteogenic and adipogenic differentiation is upset by several clinical diseases. The shift in BMSCs differentiation from osteogenesis to adipogenesis, can impede bone production and increase the accumulation of adipocytes in various pathological conditions associated with bone loss, like osteoporosis.[25]

Various studies have shown that BMSCs-derived exosomes act as crucial components in the process of bone remodeling.[26–29] Differentially expressed long non-coding RNAs (lncRNAs) in exosomes from bone marrow stem cells (BMSCs) of postmenopausal osteoporosis (PMOP) patients and healthy postmenopausal females were found using RNA microarray technology. In exosomal BMSCs of PMOP patients, 148 of these differentially expressed lncRNAs were elevated and 138 were found to be downregulated.[30] Differentially expressed lncRNAs may target the PI3K-Akt, MAPK, and Wnt/β-catenin pathways, as demonstrated by functional studies.[30] Another study was based on the microarray analysis of circRNAs sequencing profiles from BMSCs-derived exosomes of 20 healthy post-menopausal females and PMOP patients.[31] In the study, 279 circRNAs were found to be downregulated and 237 circRNAs were observed to be upregulated. Functional analysis revealed that circRNAs were differentially expressed in patients with PMOP and take part in the control of autophagy, PI3K-Akt signaling, FoxO signaling, and MAPK signaling.[31] These findings from PMOP patients have sufficiently proved that the significance of BMSCs-derived exosomes on osteogenic differentiation, suggesting potential clinical application of BMSCs-derived exosomes in osteoporosis.

Exosomes generated from bone marrow microglia (BMSCs) that carry particular lncRNAs or miRNAs support osteogenic differentiation (Figure 2). Thus, targeting lncRNAs or miRNAs in exosomes could be a novel approach to treating osteoporosis. Exosomes produced from the femoral bone marrow of patients who suffered trauma were isolated. Exosomal MALAT1, produced by BMSCs, sponged miR-34c in osteoblasts (hFOB1.19) cells, increasing SATB2 expression and osteoblast activity.[32] In ovariectomized (OVX) mice, the expression of MALAT1 and SATB2 manifested a significant reduction, while the expression of miR-34 exhibited an increase in comparison with the control mice.[32] Upregulated MALAT1 could alleviate the symptoms of osteoporosis in mice. Thus, BMSC-Exos carrying MALAT1 could play a potential protective role in preventing osteoporosis osteoporosis.[32] The femoral bone marrow of trauma

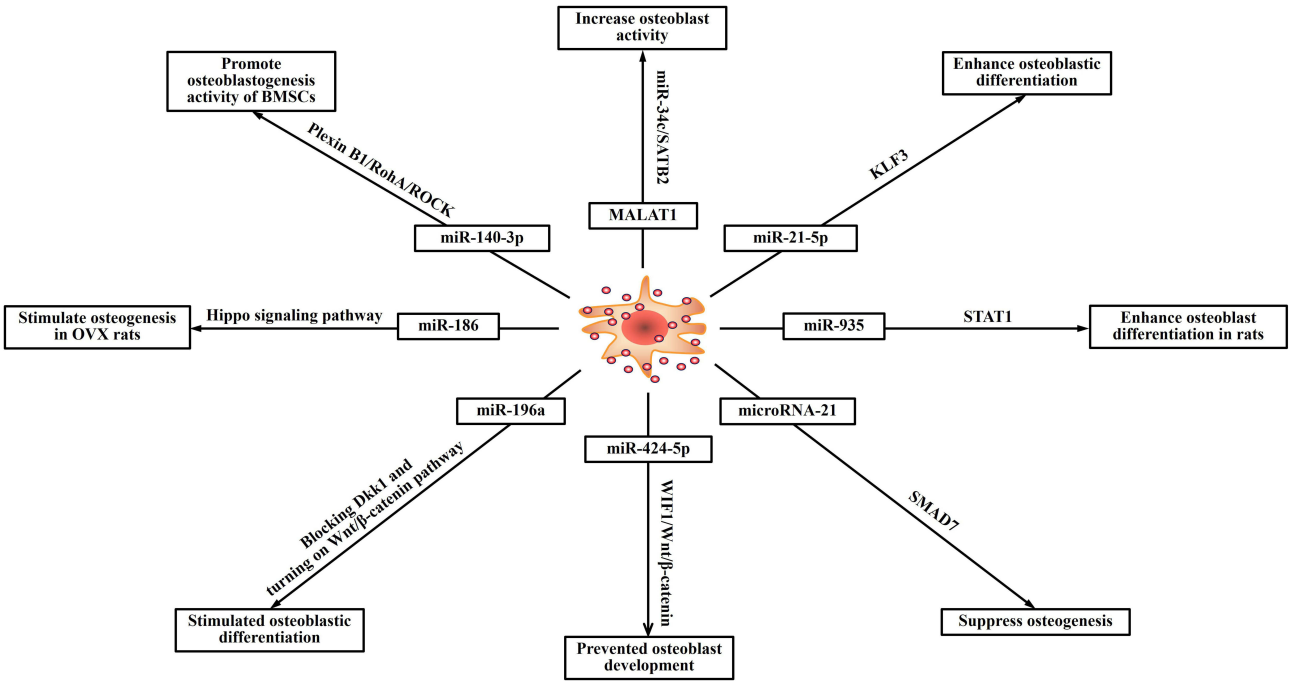

Figure 2 Mechanisms involved in osteogenic differentiation by bone marrow mesenchymal stem cells (BMSCs)-derived exosomes.

patients was employed for isolating BMSCs-derived exosomes, which were found to improve proliferation, osteoblastic differentiation, and ALP activity of human osteoblasts (hFOB1.19 cells).[33] Exosomal miR-21-5p produced from BMSCs, improved osteoporosis by mediating KLF3, enhancing osteoblastic differentiation and ALP activity of hFOB1.19 cells, and offering a possible treatment approach for osteoporosis.[33] The hFOB1.19 cells were subjected to treatment with PBS or BMSC-derived exosomes, and the differentially expressed miRNAs were examined using a Thermo Fisher GeneChipTM miRNA 4.1Array Strip microarray. When osteoblasts were treated with exosomes produced from BMSCs, the expression of miR-935 was noticeably greater among the 125 differentially expressed miRNAs.[34] By downregulating STAT1, the exosomes derived from BMSCs and containing miR-935 may enhance osteoblast proliferation and differentiation in rats, hence reducing the symptoms of osteoporosis.[34] Osteoporosis sufferers' and healthy individuals' BMSCs were separated and cultured. Using ultracentrifugation, exosomes were separated from the suspension of MSCs. Exosomes produced from MSCs that were isolated from osteoporosis patients had noticeably higher levels of MicroRNA-21 expression than those that were retrieved from healthy adults.[35] By regulation of MSC-derived exosomes isolated from osteoporosis patients via binding to SMAD7, microRNA-21 was able to suppress osteogenesis.[35] Another study investigating the exosomes produced from osteoporosis patients' BMSCs demonstrated that WIF1 was knocked down and miR-424-5p was overexpressed.[36] Exosomes overexpressing miR-424-5p blocked WIF1/Wnt/β-catenin-mediated production of OPN, OCN, Runx2, and ALP activity, which in turn prevented osteoblast development.[36] Exosomal miR-196a produced from bone marrow microSCs strongly stimulated osteoblastic differentiation by blocking Dkk1 and turning on the Wnt/β-catenin pathway.[37] Using miRNA-seq, exosomes produced from the BMSCs of OVX and normal rats were separated and identified. Compared to the OVX group, the exosomes+OVX group had a higher degree of miR-186 expression. Exosomal miR-186 may stimulate osteogenesis in OVX rats to prevent PMOP development by blocking the Hippo signaling pathway.[38] Significant reductions in miR-140-3p expression were seen in exosomes produced from ADSCs, BMSCs, and serum of diabetic rats. Through the plexin B1/RohA/ROCK signaling pathway, miR-140-3p overexpressed Exos may promote the osteoblastogenesis activity of BMSCs and speed up diabetic wound healing in diabetic rats.[39] Although the research in vitro about BMSCs-derived exosomes in osteogenic differentiation are a lot recently, whether these findings obtained can be applied to osteoporosis patients needs to be further investigated.

A growing body of research has shown that angiogenesis and osteoblast differentiation have a significant impact on the growth and development of bones. By specifically targeting VASH1, BMSC-derived exosomal miR-29a may improve endothelial cell migration, proliferation, and tube formation, thus fostering angiogenesis.[40] When compared to young BMSCs-Exo, the aged BMSCs-Exo had a significantly lower level of miR-29a, suggesting that miR-29a may be involved in age-related bone loss. By increasing the number of endothelial cells, the elderly animals treated with miR-29a-loaded BMSCs-Exos were able to increase trabecular bone mass.[40] The identification of the expression pattern of exosomal miRNAs that had been derived from bone marrow mesenchymal stem cells (BMSCs) was conducted in three experimental groups: the sham group, the ovariectomy (OVX) group, and the OVX knee loading group. The levels of miR-214-3p in the exosomes of the OVX group were found to be elevated, whereas knee loading resulted in a significant decrease in its expression. Daily application of knee loading for two weeks effectively mitigated the bone loss generated by ovariectomy (OVX). This beneficial effect was achieved through the promotion of type H vascular formation and the downregulation of exosomal miR-214-3p.[41] Osteoporosis may be caused by a decrease in angiogenesis, but it may also be alleviated by a localized increase in angiogenesis.[42] These finding have indicated that BMSCs-derived exosomes acted as a pro-angiogenic factor, which might be a new therapeutic target for the treatment of osteoporosis.

Serum-Derived Exosomes

Exosomes were collected and identified in the serum of patients with and without osteoporosis. To find out the expression profile of lncRNAs produced from exosomes in the serum of OP patients, RNA sequencing was carried out. 296 differently expressed serum exosomal lncRNAs were found to be up-regulated and 97 differentially expressed serum exosomal lncRNAs were identified to be down-regulated in the current study.[43] Differentially expressed serum exosomal lncRNAs were shown to be strongly enriched in osteoporosis-related pathways, such as those regulating insulin secretion, activating MAPK activity, cellular response to metal ions, proteolysis, and fucosylation, according to bioinformatics analysis.[43] TCONS_00072128, a newly identified lncRNA, was significantly down-regulated among them. The overexpression of LncRNA TCONS_00072128 may enhance the ability of BMSCs to differentiate into osteogenic tissues by upregulating the levels of caspase 8 and ALP.[44] A different study examined the variations in circulating miRNAs in serum exosomes among postmenopausal women who had normal bone mass and osteoporotic fractures.[45] The results of the bioinformatics study and sRNA deep sequencing revealed that serum exosomal miRNAs were expressed differently in PMOP patients who had fragility fractures. Bone mineral density (BMD) of L1-L4, femur neck, and total hip was shown to be correlated with three miRNAs (mir-324-3p, mir-766-3p, and mir-1247-5p), whereas BMD of femur neck and total hip was found to be correlated with mir-330-5p and mir-3124-5p. BMD and mir-330-5p showed a positive correlation, however mir-3124-3p manifested a negative correlation with BMD. It was discovered that mir-330-5p impeded the osteogenic differentiation of BMSCs and suppressed ALP activity, whereas mir-3124-5p had the reverse effect.[45] Furthermore, small RNA sequencing was used to elucidate the expression of miRNA in plasma exosomes among individuals with osteoporosis, osteopenia, and normal bone mass.[46] Using miRNA-mRNA KEGG networks, it was shown that miR-642a-3p, one of these differentially expressed miRNAs in plasma exosomes, is involved in bone remodeling and helps predict and diagnose early postmenopausal osteoporosis.[46] Moreover, quantitative proteomics was used to examine the protein profiles of plasma exosomes from individuals with normal bone mass and patients suffering from osteoporosis and osteopenia.[47] Between the groups, 45 distinct proteins had varying expression levels. Four of these, PSMB9, AARS, PCBP2, and VSIR, were confirmed by bioinformatics research to be related to osteoporosis.[47] Based on those studies, the PMOP-related datasets were retrieved from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), which includes the circRNA microarray dataset (GSE161361), miRNA microarray dataset (GSE64433), and mRNA microarray dataset (GSE56116). The circRNA-miRNA-TF mRNA regulation network derived from serum exosomes was established by applying the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and Gene Ontology (GO) enrichment analysis for the identification of potential important functions of differentially expressed messenger RNAs (mRNAs).[48] Quantitative proteomics analysis was used in a different investigation to identify the serum-derived exosomes of elderly patients with osteoporosis, osteopenia, and normal volunteers.[49] Normal volunteers with osteopenia's serum-derived exosomes stimulated osteoblast development in vitro, but osteoporosis sufferers' serum-derived exosomes inhibited osteoblast formation in osteogenesis.[49] Following fatigue

loading, the osteogenic differentiation potential and proliferation of bone marrow stem cells (BMSCs) extracted from elderly osteoporotic rats were reduced.[50] In the fatigue-loading group of old osteoporotic rats treated with serum exosomes from young rats, the BMSCs of the rats partially promoted osteogenic differentiation by upregulating the expression of miRNA-19b-3p.[50] The serum exosomes from lipopolysaccharide (LPS)-induced mice were increased.[51] In LPS-induced mice, the serum exosomal miRNAs were found to be differentially expressed in comparison with the control group.[51] Among these, serum exosomes and LPS-treated mice's femurs showed higher levels of miRNA-125b-5p, miRNA-132-3p, and miRNA-214-3p. It has been found that these miRNAs worsen bone loss in mice given LPS injections and prevent osteogenic development in MC3T3-E1 cells.[51] These findings suggested that serum-derived exosomes might offer viable PMOP treatment options. However, more research is needed to determine the specific mechanism of the associated network.

Human Umbilical Cord Mesenchymal Stem Cell (HucMSC)-Derived Exosomes

Exosomes generated from human umbilical cord mesenchymal stem cells (HucMSCs) enhanced osteoblast cell proliferation and differentiation.[52] In an OVX mouse osteoporosis model, HucMSC-derived exosomes could prevent osteoporosis.[52] Exosome-related miRNAs between the DOP rats and DOP+exosomes rats were identified using high-throughput miRNA sequencing to better understand the molecular mechanism of HucMSC-derived exosomes on disuse osteoporosis (DOP). In rat models of DOP produced by hind limb unloading, the results of miRNA-seq indicated that 14 miRNAs were elevated and 7 miRNAs were downregulated.[53] Among these, DOP+exosomes rats had an increase in exosomal miR-1263. Exosomal miR-1263 may stop Mob1 and the Hippo signaling pathway to reduce BMSC apoptosis and avoid rat DOP.[53] Due to researches focus on HucMSC-derived exosomes influence bone remodeling are few recently, so more in-depth researches are needed in the future.

Exosomes Derived from Adipose Tissue-Derived MSCs

Recent studies have shown that primary osteoblasts might be efficiently shielded from TNF-α-induced cytotoxicity and death by exosomes produced from adipose-derived stem cells (ADSCs-Exos).[54] By sponging miR-141-5p in primary osteoblasts, KCNQ1OT1-Exos showed a more pronounced inhibitory effect on TNF-α-induced cytotoxicity and apoptosis than ADSCs-Exos.[54] MiR-21 overexpressing adipose tissue-derived MSCs (AD-MSCs) could produce exosomes that upregulate interleukin (IL)-6 expression in the spine, decrease the number of osteoclasts, decrease the content of deoxypyridinoline in the urine, tartrate-resistant acid phosphatase (TRACP)-5b, and cathepsin K in the serum, and increase bone mineral content and BMD. These effects would alleviate osteoporosis in ankylosing spondylitis (AS) mice.[55]

Endothelial Cells-Derived Exosomes

One of the negative effects of long-term high-dose glucocorticoids (GCs) is decreased bone mineral density (BMD) and bone microstructure degradation. The most prevalent kind of secondary osteoporosis is caused by GCs.[56] Recently identified as a form of controlled cell death, ferroptosis is closely associated with lipid peroxidation and bone development in GC-induced osteoporosis.[57] In MC3T3-E1 cells and mice, endothelial cell-secreted exosomes (EC-Exos) have the potential to cure GCs-induced osteoporosis by preventing apoptosis and ferritinophagy-dependent ferroptosis.[57]

Platelet Lysate Exosomes

Platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), and basic fibroblast growth factor (bFGF) were the main components of platelet lysates (PL), which had a strong pro-regeneration and pro-angiogenesis effect in wound healing and local bone healing.[58,59] After isolating PL-derived exosomes (PL-exo) to enhance platelet-derived growth factors (GFs), DSPE-PEG-grafted alendronate (ALN) conjugated PL-exo exosomes (PL-exo-ALN) to create bone-targeting delivery of platelet lysate exosomes (PL-exo-ALN).[60] This work showed that PL-exo-ALN could reverse GCs and that it prevented the angiogenesis of BMSCs and endothelial progenitor cells (EPCs) in vitro.[60] Furthermore, through promoting bone anabolism and angiogenesis in rats, intravenous injection of PL-exo-ALN might also effectively reverse GCs-induced osteoporosis (GIOP).[60]

Macrophages-Derived Exosomes

Two types of macrophages (Mφs) exist that are master regulators of immune responses: the pro-inflammatory M1 type and the anti-inflammatory M2 type.[61] Treatment with M1Mφ-derived exosomes inhibited the osteogenic differentiation of MC3T3-E1 cells and worsened the bone loss caused by OVX in mice.[62] Following treatment with M1Mφ-derived exosomes, there was a significant rise in the expression of miR-98 in both OVX mice's bone tissues and MC3T3-E1 cells.[62] Through increased exosomal miR-98, DUSP1 downregulation, and JNK activation, the M1Mφ-derived exosome therapy may exacerbate bone loss and worsen bone function.[62]

Osteoblast-Derived Exosomes

Differential effects were observed in the metabolism and osteogenic development of BMSCs when EVs were extracted from osteoblasts of patients with hip OA (coxarthrosis/CA), osteoporosis (OP), or a combination of both conditions (CA/OP).[63] In a different investigation, exosomes generated from senescent osteoblasts showed increased expression of miR-139-5p. By targeting TBX1, senescent osteoblast-derived exosomal miR-139-5p may stimulate senescence and prevent endothelial cell growth.[64]

Other Cells-Derived Exosomes

T-cell exosomes were derived from non-osteoporotic and osteoporotic postmenopausal women.[65] T cell exosomes derived from osteoporotic people may negatively impact osteoblastic function by lowering genes linked to osteoblasts, such as Runx2, osteocalcin, type I collagen, and osteopontin.[65] By releasing miRNA-30a and miRNA-23a, systemic mastocytosis (SM)-derived exosomes (SM-EVs) with a mast cell signature and exosomes derived from neoplastic mast cells could inhibit the differentiation of preosteoblastic cells into osteoblasts, hence limiting osteoblastogenesis and bone production.[66] When SM-EVs were injected into mice, they also reduced the expression of osteoblast markers, as well as the volume and microarchitecture of trabecular bone.[66]

Above these findings indicated that exosomes are closely connected with osteogenic differentiation, thus it has great clinical significance to do comprehensive investigation to develop effective therapeutic approaches for osteoporosis.

The Potential Therapeutic Effects of EVs in Osteoclast Differentiation

Exosomes produced from BMSCs treated with cyclic mechanical stretch (CMS) efficiently inhibited osteoclastogenesis in vitro by blocking the nuclear factor kappa-B (NF-κB) signaling pathway triggered by RANKL.[67] Tarrate-resistant acid phosphatase (TRAP)-positive osteoclasts were dramatically reduced in quantity when treated with exosomes produced from CMS-treated BMSCs.[67] In hindlimb unloading (HU)-induced severe DOP mice, exosomes obtained from CMS-treated BMSCs and static-cultured BMSC-derived exosomes (static_Exos) both further prevented osteoporosis.[67] It has been shown that exosomes released by vascular endothelial cells (EC) have a better ability to target bone. EC-Exos may considerably restrict osteoclast differentiation and function in vitro by reducing the area of bone resorption and inhibiting actin ring formation in mature osteoclasts.[68] EC-Exos were injected intraperitoneally into ovariectomized mice twice a week. In OVX mice, EC-Exos may decrease bone mass (BMD), bone volume/total volume (BV/TV), and unorganized trabecular architecture (Tb. N).[68] Using gene sequencing, the differentially expressed miRNAs in the control group and EC-Exos-treated bone marrow macrophages (BMMs) were found. EC-Exos, one of these miRNAs, may prevent osteoclastic bone resorption in part by supplying macrophages with miR-155.[68] BMSC-derived exosomes from aged rats had significantly greater levels of exosomal miR-31a-5p than exosomes from younger rats.[69] Older rats' BMSC-derived exosomal miR-31a-5p decreased osteoblastogenesis by promoting the development of senescence-associated heterochromatin foci (SAHF).[69] BMSCs derived Exosomal miR-31a-5p inhibited RhoA activity to increase osteoclastogenesis and bone resorption in old rats.[69] Treatment with antagomiR-31a-5p prevented bone loss in old mice by drastically reducing osteoclast numbers and osteoclastic differentiation.[69] Adipose-derived mesenchymal stem cells (AD-MSCs) have the ability to inhibit pro-inflammatory cytokines (IL-1b and IL-18) that are generated by high glucose levels in osteoclasts.[70] By reducing RANKL expression and the ratio of RANKL/OPG, ADSCs-exo may counteract hypoxia and serum deprivation (H/SD)-induced osteocyte death and osteocyte-mediated osteoclastogenesis in the osteocyte-like

cell line MLO-Y4.[71] By inhibiting NLRP3 inflammasome activation, AD-MSCs were also able to decrease bone resorption and reverse bone loss in rats with diabetic osteoporosis produced by streptozotocin.[70] Exosomes from AD-MSCs overexpressing microRNA-146a were able to suppress the expression of pro-inflammatory cytokines (TNF-a, IL-18, and IL-1b) in high glucose-treated osteoclasts.[72] Exosomes from AD-MSCs overexpressing microRNA-146a were able to reduce bone resorption and reverse the effects of diabetic-induced bone loss in rats with streptozotocin-induced diabetic osteoporosis.[72] Exosomes may offer prospective therapeutic targets in osteoclast differentiation as demonstrated by these findings in animals and cells. Therefore, there is an urgent need to do intensive investigations in osteoporosis patients to fill the clinical gaps.

Effects of Traditional Chinese Medicine on Exosomes

According to recent studies, osteoporosis was lessened by using bone marrow-derived mesenchymal stem cell-derived exosomes (BMSC-Exos), which may represent a new osteoporosis treatment target. Traditional Chinese medicine treated BMSC-Exos. Traditional Chinese medicine produced artesunate (ART), a derivative of artemisinin, which is commonly used to treat malaria.[73] Recent research has demonstrated that antiretroviral therapy (ART) may have preventive effects against osteoporosis by modulating the miR-503/RANK axis, inhibiting the MAPK and AKT pathways, and decreasing osteoclastogenesis and osteoclast activities in vitro.[74] Another study revealed that ART-treated BMSC-Exos enhanced hFOB1.19 cells' osteoblast activity by upregulating osteogenesis-related molecules (including RUNX2, BMP2, and ATF4) and increasing alkaline phosphatase activity. This was achieved by providing SNHG7 by altering the TAF15/RUNX2 axis.[75] ART-treated BMSC-Exos also attenuated osteoporosis in OVX mice.[75] Another study revealed that ART-treated BMSC-Exos enhanced hFOB1.19 cells' osteoblast activity by upregulating osteogenesis-related molecules (including RUNX2, BMP2, and ATF4) and increasing alkaline phosphatase activity. This was achieved by providing SNHG7 by altering the TAF15/RUNX2 axis.[76] The primary chemical ingredient isolated from *Morinda officinalis* (MO) is called Morinda officinalis polysaccharide (MOP), and it has anti-osteoporotic properties.[76] MOP treatment significantly prevented OVX-induced bone loss in rats by decreasing levels of bone turnover markers and trabecular microarchitecture degradation.[77] Additionally, a recent study demonstrated that MOP therapy helped alleviate the symptoms of osteoporosis in rats with GIOP by promoting osteoblastic differentiation and suppressing osteoclastic differentiation.[78] Osteoclast differentiation was induced in BMMs. Osteoclastic differentiation and the development of BMMs generated by RANKL were dramatically enhanced when BMSC-Exo isolated from GIOP rats were treated. The osteoclastic differentiation and proliferation of BMMs may be effectively reduced by treating BMSC-Exo from MOP-treated GIOP rats (MOP-Exo) via upregulating prostaglandin-endoperoxide synthase 2 (PTGS2) and downregulating miR-101-3p.[78] Bovine colostrum-derived exosomes inhibited osteoclast differentiation in Raw264.7 cells.[79] In mice with glucocorticoid-induced osteoporosis, oral administration of exosomes produced from bovine colostrum for two months markedly increased bone mineral density via improvement in the composition of gut microbiota.[79] These studies about Chinese medicine were mainly from cells and animals, whether the findings hold true in humans need further study.

Future Perspectives

An increasing amount of research has demonstrated that exosomes are crucial in mediating osteoporosis. Nonetheless, further research should be done on exosome-based bone-targeting delivery methods to prevent pathological bone loss. An engineered exosome delivery system called BT-Exo-siShn3 was developed in a study using exosomes from iMSCs, which are derivatives of induced pluripotent stem cells (iPSCs). After the exosomes were loaded with siRNA of Shn3 using electroporation, the bone-targeting peptide modified with a diacyllipid tail was fixed onto the exosome membrane via hydrophobic interaction.[80] Because BT-Exo-siShn3 could preferentially transport siShn3 to osteoblasts, it improved osteogenic differentiation by promoting the development of type H vasculature and vascularization by upregulating SLIT3 production.[80] Genetically engineered NIH-3T3 cells derived CXCR4+ exosomes that aggregated in the bone marrow with selectivity. Hybrid nanoparticles were created by fusing liposomes containing antagomir-188 with CXCR4+ exosomes (NPs).[81] The hybrid NPs stimulated osteogenesis and inhibited BMSC adipogenesis. They particularly congregated in the bone marrow and released antagomir-188. Antagonist-188-loaded hybrid nanoparticles may be able to stop age-related bone loss by improving the quality and porosity of cortical bone.[81] However, the complicated

mechanism between exosomes and bone remodeling is still not fully understood. Therefore, we need to spare no effort to do related clinical application and looking for an effective treatment for osteoporosis.

Conclusion

Nearly all cell types secrete exosomes, which are made up of the origin cells' lipids, proteins, and nucleic acids. We reviewed the biology of exosomes and their crucial functions in osteoporosis in this study. Exosomes function as highly effective biomolecule transporters inside the milieu of bone remodeling. According to preclinical research, exosomes contribute to bone homeostasis by controlling osteoblast and osteoclast development and activity. Exosomes are anticipated to emerge as a dependable novel treatment option for osteoporosis due to their remarkable therapeutic potential. However, this research in this realm is still in its initial stages and the effect on humans is not yet fully understood. As a result, there are still obstacles to overcome before exosomes' therapeutic potential may be fully explored.

Disclosure

Yanxia Chen and Yinxi He declare that they have no conflicts of interest in this work.

References

1. Tkach M, Théry C. Communication by extracellular vesicles: where we are and where we need to go. *Cell*. 2016;164:1226–1232. doi:10.1016/j.cell.2016.01.043
2. Baek G, Choi H, Kim Y, Lee HC, Choi C. Mesenchymal stem cell-derived extracellular vesicles as therapeutics and as a drug delivery platform. *Stem Cells Transl Med*. 2019;8:880–886. doi:10.1002/sctm.18-0226
3. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci*. 2019;9:19. doi:10.1186/s13578-019-0282-2
4. Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. *Hum Mol Genet*. 2012;21:R125–134. doi:10.1093/hmg/dds317
5. Shao H, Im H, Castro CM, Breakefield X, Weissleder R, Lee H. New technologies for analysis of extracellular vesicles. *Chem Rev*. 2018;118:1917–1950. doi:10.1021/acs.chemrev.7b00534
6. Trams EG, Lauter CJ, Salem N, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim Biophys Acta*. 1981;645:63–70. doi:10.1016/0005-2736(81)90512-5
7. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem*. 1987;262:9412–9420. doi:10.1016/S0021-9258(18)48095-7
8. He C, Zheng S, Luo Y, Wang B. Exosome theranostics: biology and translational medicine. *Theranostics*. 2018;8:237–255. doi:10.7150/thno.21945
9. Yang Z, Zhang W, Ren X, Tu C, Li Z. Exosomes: a friend or foe for osteoporotic fracture? *Front Endocrinol*. 2021;12:679914. doi:10.3389/fendo.2021.679914
10. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367. doi:10.1126/science.aau6977
11. Ferguson SW, Nguyen J. Exosomes as therapeutics: the implications of molecular composition and exosomal heterogeneity. *J Control Release*. 2016;228:179–190. doi:10.1016/j.jconrel.2016.02.037
12. Liu Y, Wang Y, Lv Q, Li X. Exosomes: from garbage bins to translational medicine. *Int J Pharm*. 2020;583:119333. doi:10.1016/j.ijpharm.2020.119333
13. Simons M, Raposo G. Exosomes–vesicular carriers for intercellular communication. *Curr Opin Cell Biol*. 2009;21:575–581. doi:10.1016/j.ceb.2009.03.007
14. S ELA, Mäger I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov*. 2013;12:347–357. doi:10.1038/nrd3978
15. Katsimbri P. The biology of normal bone remodelling. *Eur J Cancer Care*. 2017;2017:26.
16. Lane JM, Russell L, Khan SN. Osteoporosis. *Clin Orthop Relat Res*. 2000;139–150. doi:10.1097/00003086-200003000-00016
17. Meng F, Xue X, Yin Z, Gao F, Wang X, Geng Z. Research progress of exosomes in bone diseases: mechanism, diagnosis and therapy. *Front Bioeng Biotechnol*. 2022;10:866627. doi:10.3389/fbioe.2022.866627
18. Wang Y, Lin Q, Zhang H, et al. M2 macrophage-derived exosomes promote diabetic fracture healing by acting as an immunomodulator. *Bioact Mater*. 2023;28:273–283. doi:10.1016/j.bioactmat.2023.05.018
19. Seeman E. Bone modeling and remodeling. *Crit Rev Eukaryot Gene Expr*. 2009;19:219–233. doi:10.1615/CritRevEukarGeneExpr.v19.i3.40
20. Lee JY, Yang JY, Kim SW. Bone lining cells could be sources of bone marrow adipocytes. *Front Endocrinol*. 2021;12:766254. doi:10.3389/fendo.2021.766254
21. Ducy P, Schinke T, Karsenty G. The osteoblast: a sophisticated fibroblast under central surveillance. *Science*. 2000;289:1501–1504. doi:10.1126/science.289.5484.1501
22. Bar-Shavit Z. The osteoclast: a multinucleated, hematopoietic-origin, bone-resorbing osteoimmune cell. *J Cell Biochem*. 2007;102(5):1130–1139. doi:10.1002/jcb.21553
23. Ono T, Hayashi M, Sasaki F, Nakashima T. RANKL biology: bone metabolism, the immune system, and beyond. *Inflamm Regen*. 2020;40:2. doi:10.1186/s41232-019-0111-3
24. Quinn JM, Gillespie MT. Modulation of osteoclast formation. *Biochem Biophys Res Commun*. 2005;328:739–745. doi:10.1016/j.bbrc.2004.11.076

25. Rosen CJ, Ackert-Bicknell C, Rodriguez JP, Pino AM. Marrow fat and the bone microenvironment: developmental, functional, and pathological implications. *Crit Rev Eukaryot Gene Expr*. 2009;19:109–124. doi:10.1615/CritRevEukarGeneExpr.v19.i2.20
26. Sadat-Ali M, Al-Dakheel DA, Al-Turki HA, Acharya S. Efficacy of autologous bone marrow derived Mesenchymal stem cells (MSCs), osteoblasts and osteoblasts derived exosome in the reversal of ovariectomy (OVX) induced osteoporosis in rabbit model. *Am J Transl Res*. 2021;13:6175–6181.
27. Luo ZW, Li FX, Liu YW, et al. Aptamer-functionalized exosomes from bone marrow stromal cells target bone to promote bone regeneration. *Nanoscale*. 2019;11:20884–20892. doi:10.1039/C9NR02791B
28. Zhao P, Xiao L, Peng J, Qian YQ, Huang CC. Exosomes derived from bone marrow mesenchymal stem cells improve osteoporosis through promoting osteoblast proliferation via MAPK pathway. *Eur Rev Med Pharmacol Sci*. 2018;22:3962–3970. doi:10.26355/eurrev_201806_15280
29. Xie Y, Hu JH, Wu H, Huang ZZ, Yan HW, Shi ZY. Bone marrow stem cells derived exosomes improve osteoporosis by promoting osteoblast proliferation and inhibiting cell apoptosis. *Eur Rev Med Pharmacol Sci*. 2019;23:1214–1220. doi:10.26355/eurrev_201902_17014
30. Wang J, Fu M, He S, Cai P, Xiang X, Fang L. Expression profile analysis of lncRNA in bone marrow mesenchymal stem cells exosomes of postmenopausal osteoporosis patients through microarray and bioinformatics analyses. *Pathol Res Pract*. 2022;236:153985. doi:10.1016/j.prp.2022.153985
31. Fu M, Fang L, Xiang X, Fan X, Wu J, Wang J. Microarray analysis of circRNAs sequencing profile in exosomes derived from bone marrow mesenchymal stem cells in postmenopausal osteoporosis patients. *J Clin Lab Anal*. 2022;36:e23916. doi:10.1002/jcla.23916
32. Yang X, Yang J, Lei P, Wen T. LncRNA MALAT1 shuttled by bone marrow-derived mesenchymal stem cells-secreted exosomes alleviates osteoporosis through mediating microRNA-34c/SATB2 axis. *Aging*. 2019;11:8777–8791. doi:10.18632/aging.102264
33. You M, Ai Z, Zeng J, Fu Y, Zhang L, Wu X. Bone mesenchymal stem cells (BMSCs)-derived exosomal microRNA-21-5p regulates Kruppel-like factor 3 (KLF3) to promote osteoblast proliferation in vitro. *Bioengineered*. 2022;13:11933–11944. doi:10.1080/21655979.2022.2067286
34. Zhang Y, Cao X, Li P, et al. microRNA-935-modified bone marrow mesenchymal stem cells-derived exosomes enhance osteoblast proliferation and differentiation in osteoporotic rats. *Life Sci*. 2021;272:119204. doi:10.1016/j.lfs.2021.119204
35. Jiang LB, Tian L, Zhang CG. Bone marrow stem cells-derived exosomes extracted from osteoporosis patients inhibit osteogenesis via microRNA-21/SMAD7. *Eur Rev Med Pharmacol Sci*. 2018;22:6221–6229. doi:10.26355/eurrev_201810_16028
36. Wei Y, Ma H, Zhou H, et al. miR-424-5p shuttled by bone marrow stem cells-derived exosomes attenuates osteogenesis via regulating WIF1-mediated Wnt/β-catenin axis. *Aging*. 2021;13:17190–17201. doi:10.18632/aging.203169
37. Yang K, Li C, Lu S, et al. Exosomes from bone marrow mesenchymal stem cells promoted osteogenic differentiation by delivering miR-196a that targeted Dickkopf-1 to activate Wnt/β-catenin pathway. *Bioengineered*. 2021;12:6115–6133. doi:10.1080/21655979.2021.1972644
38. Li L, Zhou X, Zhang JT, et al. Exosomal miR-186 derived from BMSCs promote osteogenesis through hippo signaling pathway in postmenopausal osteoporosis. *J Orthop Surg Res*. 2021;16:23. doi:10.1186/s13018-020-02160-0
39. Wang N, Liu X, Tang Z, et al. Increased BMSC exosomal miR-140-3p alleviates bone degradation and promotes bone restoration by targeting Plxnb1 in diabetic rats. *J Nanobiotechnology*. 2022;20:97. doi:10.1186/s12951-022-01267-2
40. Lu GD, Cheng P, Liu T, Wang Z. BMSC-derived exosomal miR-29a promotes angiogenesis and osteogenesis. *Front Cell Dev Biol*. 2020;8:608521. doi:10.3389/fcell.2020.608521
41. Wang X, Li X, Li J, et al. Mechanical loading stimulates bone angiogenesis through enhancing type H vessel formation and downregulating exosomal miR-214-3p from bone marrow-derived mesenchymal stem cells. *FASEB J*. 2021;35:e21150. doi:10.1096/fj.202001080RR
42. Tong X, Chen X, Zhang S, et al. The effect of exercise on the prevention of osteoporosis and bone angiogenesis. *Biomed Res Int*. 2019;2019:8171897. doi:10.1155/2019/8171897
43. Teng Z, Zhu Y, Zhang X, Teng Y, Lu S. Osteoporosis is characterized by altered expression of exosomal long non-coding RNAs. *Front Genet*. 2020;11:566959. doi:10.3389/fgene.2020.566959
44. Yang Y, Miao L, Chang S, et al. Exosome-Derived LncRNA TCONS_00072128 mediated osteogenic differentiation and inflammation by caspase 8 regulation. *Front Genet*. 2021;12:831420. doi:10.3389/fgene.2021.831420
45. Shi H, Jiang X, Xu C, Cheng Q. MicroRNAs in serum exosomes as circulating biomarkers for postmenopausal osteoporosis. *Front Endocrinol*. 2022;13:819056. doi:10.3389/fendo.2022.819056
46. Kong D, Chen T, Zheng X, Yang T, Zhang Y, Shao J. Comparative profile of exosomal microRNAs in postmenopausal women with various bone mineral densities by small RNA sequencing. *Genomics*. 2021;113:1514–1521. doi:10.1016/j.ygeno.2021.03.028
47. Chen M, Li Y, Lv H, Yin P, Zhang L, Tang P. Quantitative proteomics and reverse engineer analysis identified plasma exosome derived protein markers related to osteoporosis. *J Proteomics*. 2020;228:103940. doi:10.1016/j.jprot.2020.103940
48. Dong Q, Han Z, Tian L. Identification of serum exosome-derived circRNA-miRNA-TF-mRNA regulatory network in postmenopausal osteoporosis using bioinformatics analysis and validation in peripheral blood-derived mononuclear cells. *Front Endocrinol*. 2022;13:899503. doi:10.3389/fendo.2022.899503
49. Xie Y, Gao Y, Zhang L, Chen Y, Ge W, Tang P. Involvement of serum-derived exosomes of elderly patients with bone loss in failure of bone remodeling via alteration of exosomal bone-related proteins. *Aging Cell*. 2018;17:e12758. doi:10.1111/acel.12758
50. Xun J, Li C, Liu M, et al. Serum exosomes from young rats improve the reduced osteogenic differentiation of BMSCs in aged rats with osteoporosis after fatigue loading in vivo. *Stem Cell Res Ther*. 2021;12:424. doi:10.1186/s13287-021-02449-9
51. Wang Y, Zhang L, Wang K, et al. Circulating exosomes from mice with LPS-induced bone loss inhibit osteoblast differentiation. *Calcif Tissue Int*. 2022;111:185–195. doi:10.1007/s00223-022-00977-x
52. Yahao G, Xinjia W. The role and mechanism of exosomes from umbilical cord mesenchymal stem cells in inducing osteogenesis and preventing osteoporosis. *Cell Transplant*. 2021;30:9636897211057465. doi:10.1177/09636897211057465
53. Yang BC, Kuang MJ, Kang JY, Zhao J, Ma JX, Ma XL. Human umbilical cord mesenchymal stem cell-derived exosomes act via the miR-1263/Mob1/Hippo signaling pathway to prevent apoptosis in disuse osteoporosis. *Biochem Biophys Res Commun*. 2020;524:883–889. doi:10.1016/j.bbrc.2020.02.001
54. Wang SZ, Jia J, Chen CH. lncRNA-KCNQ1OT1: a potential target in exosomes derived from adipose-derived stem cells for the treatment of osteoporosis. *Stem Cells Int*. 2021;2021:7690006. doi:10.1155/2021/7690006
55. Hu L, Guan Z, Tang C, Li G, Wen J. Exosomes derived from microRNA-21 overexpressed adipose tissue-derived mesenchymal stem cells alleviate spine osteoporosis in ankylosing spondylitis mice. *J Tissue Eng Regen Med*. 2022;16:634–642. doi:10.1002/term.3304
56. Chen M, Fu W, Xu H et al. Pathogenic mechanisms of glucocorticoid-induced osteoporosis. *Cytokine Growth Factor Rev*. 2023;70:54–66. doi:10.1016/j.cytogfr.2023.03.002.

57. Yang RZ, Xu WN, Zheng HL, et al. Involvement of oxidative stress-induced annulus fibrosus cell and nucleus pulposus cell ferroptosis in intervertebral disc degeneration pathogenesis. *J Cell Physiol*. 2021;236:2725–2739. doi:10.1002/jcp.30039
58. Oliveira SM, Pirraco RP, Marques AP, et al. Platelet lysate-based pro-angiogenic nanocoatings. *Acta Biomater*. 2016;32:129–137. doi:10.1016/j.actbio.2015.12.028
59. Xia W, Li H, Wang Z, et al. Human platelet lysate supports ex vivo expansion and enhances osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. *Cell Biol Int*. 2011;35:639–643. doi:10.1042/CBI20100361
60. Zheng G, Ma HW, Xiang GH, et al. Bone-targeting delivery of platelet lysate exosomes ameliorates glucocorticoid-induced osteoporosis by enhancing bone-vessel coupling. *J Nanobiotechnology*. 2022;20:220. doi:10.1186/s12951-022-01400-1
61. Kong L, Wang Y, Smith W, Hao D. Macrophages in bone homeostasis. *Curr Stem Cell Res Ther*. 2019;14:474–481. doi:10.2174/1574888X14666190214163815
62. Yu L, Hu M, Cui X, et al. M1 macrophage-derived exosomes aggravate bone loss in postmenopausal osteoporosis via a microRNA-98/DUSP1/JNK axis. *Cell Biol Int*. 2021;45:2452–2463. doi:10.1002/cbin.11690
63. Niedermair T, Lukas C, Li S, et al. Influence of extracellular vesicles isolated from osteoblasts of patients with cox-arthrosis and/or osteoporosis on metabolism and osteogenic differentiation of BMSCs. *Front Bioeng Biotechnol*. 2020;8:615520. doi:10.3389/fbioe.2020.615520
64. Lu Q, Qin H, Tan H, et al. Senescence osteoblast-derived exosome-mediated miR-139-5p regulates endothelial cell functions. *Biomed Res Int*. 2021;2021:5576023. doi:10.1155/2021/5576023
65. Omidvar MH, Soltani-Zangbar MS, Zamani M, et al. The effect of osteoporotic and non-osteoporotic individuals' T cell-derived exosomes on osteoblast cells' bone remodeling related genes expression and alkaline phosphatase activity. *BMC Res Notes*. 2022;15:272. doi:10.1186/s13104-022-06139-4
66. Kim DK, Bandara G, Cho YE, et al. Mastocytosis-derived extracellular vesicles deliver miR-23a and miR-30a into pre-osteoblasts and prevent osteoblastogenesis and bone formation. *Nat Commun*. 2021;12:2527. doi:10.1038/s41467-021-22754-4
67. Xiao F, Zuo B, Tao B, et al. Exosomes derived from cyclic mechanical stretch-exposed bone marrow mesenchymal stem cells inhibit RANKL-induced osteoclastogenesis through the NF-κB signaling pathway. *Ann Transl Med*. 2021;9:798. doi:10.21037/atm-21-1838
68. Song H, Li X, Zhao Z, et al. Reversal of osteoporotic activity by endothelial cell-secreted bone targeting and biocompatible exosomes. *Nano Lett*. 2019;19:3040–3048. doi:10.1021/acs.nanolett.9b00287
69. Xu R, Shen X, Si Y, et al. MicroRNA-31a-5p from aging BMSCs links bone formation and resorption in the aged bone marrow microenvironment. *Aging Cell*. 2018;17:e12794. doi:10.1111/acel.12794
70. Zhang L, Wang Q, Su H, Cheng J. Exosomes from adipose derived mesenchymal stem cells alleviate diabetic osteoporosis in rats through suppressing NLRP3 inflammasome activation in osteoclasts. *J Biosci Bioeng*. 2021;131:671–678. doi:10.1016/j.jbiosc.2021.02.007
71. Ren L, Song ZJ, Cai QW, et al. Adipose mesenchymal stem cell-derived exosomes ameliorate hypoxia/serum deprivation-induced osteocyte apoptosis and osteocyte-mediated osteoclastogenesis in vitro. *Biochem Biophys Res Commun*. 2019;508:138–144. doi:10.1016/j.bbrc.2018.11.109
72. Zhang L, Wang Q, Su H, Cheng J. Exosomes from adipose tissues derived mesenchymal stem cells overexpressing MicroRNA-146a alleviate diabetic osteoporosis in rats. *Cell Mol Bioeng*. 2022;15:87–97. doi:10.1007/s12195-021-00699-4
73. Kouakou YI, Tod M, Leboucher G, et al. Systematic review of artesunate pharmacokinetics: implication for treatment of resistant malaria. *Int J Infect Dis*. 2019;89:30–44. doi:10.1016/j.ijid.2019.08.030
74. Huang MZ, Zhuang Y, Ning X, Zhang H, Shen ZM, Shang XW. Artesunate inhibits osteoclastogenesis through the miR-503/RANK axis. *Biosci Rep*. 2020;40. doi:10.1042/BSR20194387
75. Huang MZ, Chen HY, Peng GX, et al. Exosomes from artesunate-treated bone marrow-derived mesenchymal stem cells transferring SNHG7 to promote osteogenesis via TAF15-RUNX2 pathway. *Regener Med*. 2022;17:819–833. doi:10.2217/rme-2022-0065
76. Zhang JH, Xin HL, Xu YM, et al. Morinda officinalis How. - A comprehensive review of traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol*. 2018;213:230–255. doi:10.1016/j.jep.2017.10.028
77. Zhang D, Zhang S, Jiang K, Li T, Yan C. Bioassay-guided isolation and evaluation of anti-osteoporotic polysaccharides from Morinda officinalis. *J Ethnopharmacol*. 2020;261:113113.
78. Wu P, Jiao F, Huang H, et al. Morinda officinalis polysaccharide enable suppression of osteoclastic differentiation by exosomes derived from rat mesenchymal stem cells. *Pharm Biol*. 2022;60:1303–1316. doi:10.1080/13880209.2022.2093385
79. Yun B, Maburutse BE, Kang M, et al. Short communication: dietary bovine milk-derived exosomes improve bone health in an osteoporosis-induced mouse model. *J Dairy Sci*. 2020;103:7752–7760. doi:10.3168/jds.2019-17501
80. Cui Y, Guo Y, Kong L, et al. A bone-targeted engineered exosome platform delivering siRNA to treat osteoporosis. *Bioact Mater*. 2022;10:207–221. doi:10.1016/j.bioactmat.2021.09.015
81. Hu Y, Li X, Zhang Q, et al. Exosome-guided bone targeted delivery of Antagomir-188 as an anabolic therapy for bone loss. *Bioact Mater*. 2021;6:2905–2913. doi:10.1016/j.bioactmat.2021.02.014